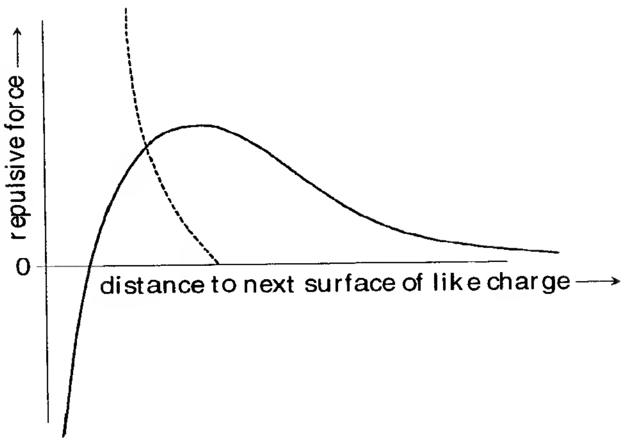

Figure 1. Steric interactions between particles are very strong , but are effective only close to the surface (dashed line); electrostatic forces can extend beyond the range of typical surface associated polymers (solid line). Here the two are shown as repulsive forces as would be found between two like surfaces during a collision. (Fitch 97).

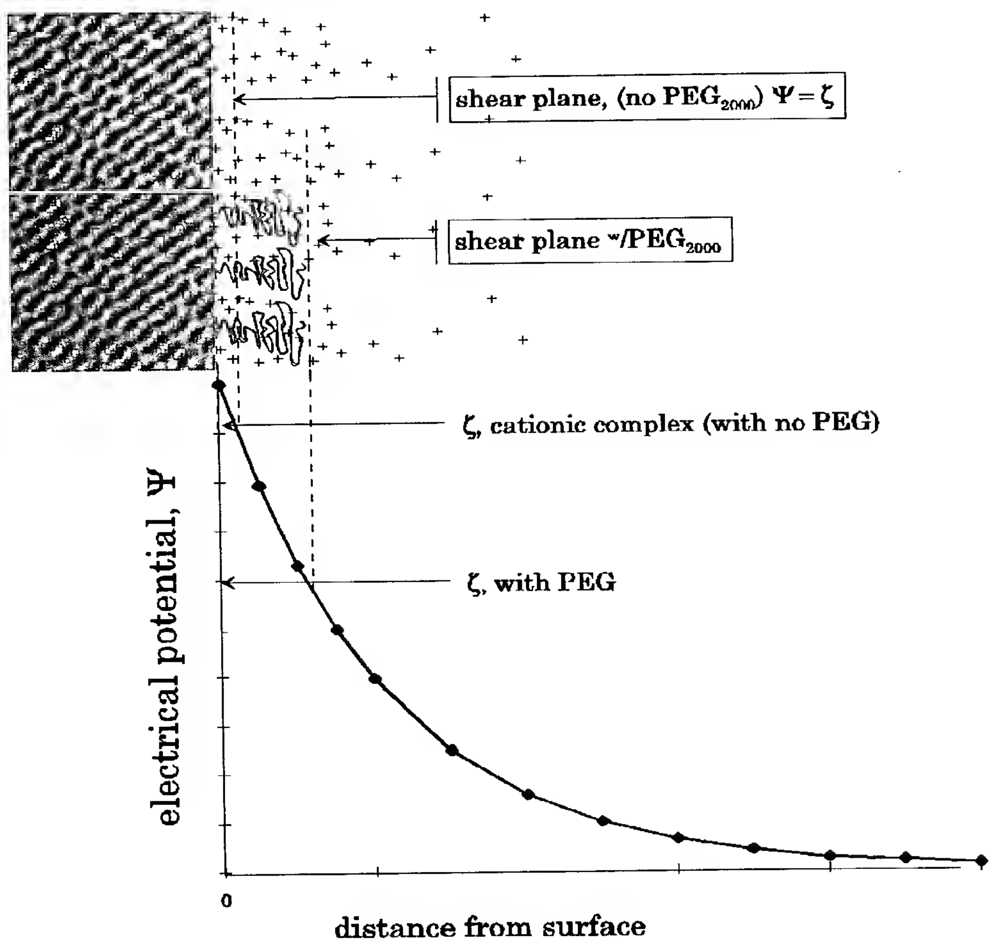

Figure 2. When ionized particles move in an electric field, they carry ions with them. The layer of carried ions adds to the hydrodynamic diameter of the particle and moves the shear plane out away from the surface. Past the shear plane, ions and solvent will not be carried by the particle. Elastic light scattering can be used to measure the mobility of charged particles in an applied electric field and from that the electrostatic potential (Ψ) at the shear plane is calculated. The magnitude of the electrostatic potential at the shear plane is called the zeta potential (ζ).

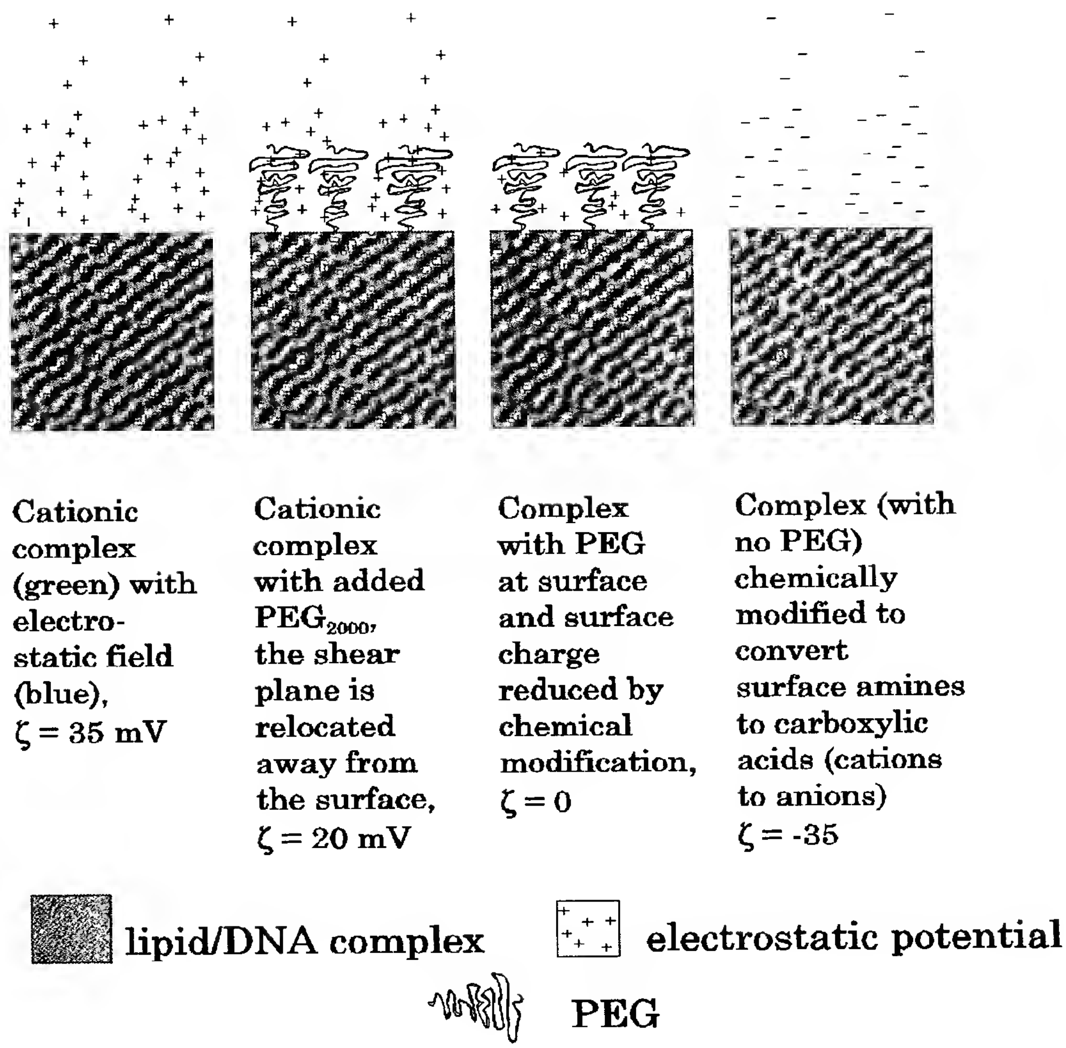

Figure 3. Surface associated polymers (like PEG2000) physically control the movement of the solution near the surface and extend the effective shear plane. The zeta potential is reduced without affecting the electrostatic field. Chemical modification of the amines at the surface of the particles can be used to alter the electrostatic field around the particle.

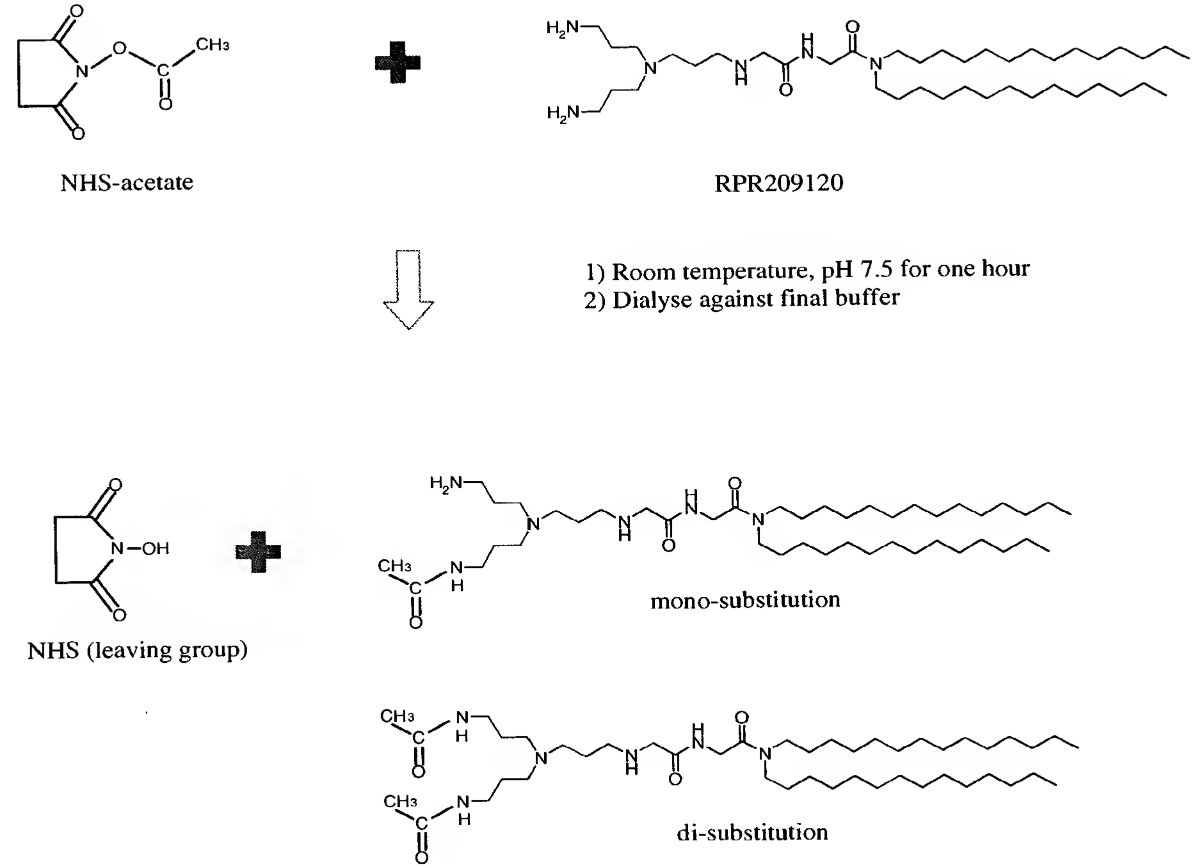

Figure 4. Chemistry of NHS acetate with the primary amine of the lipid RPR209120.

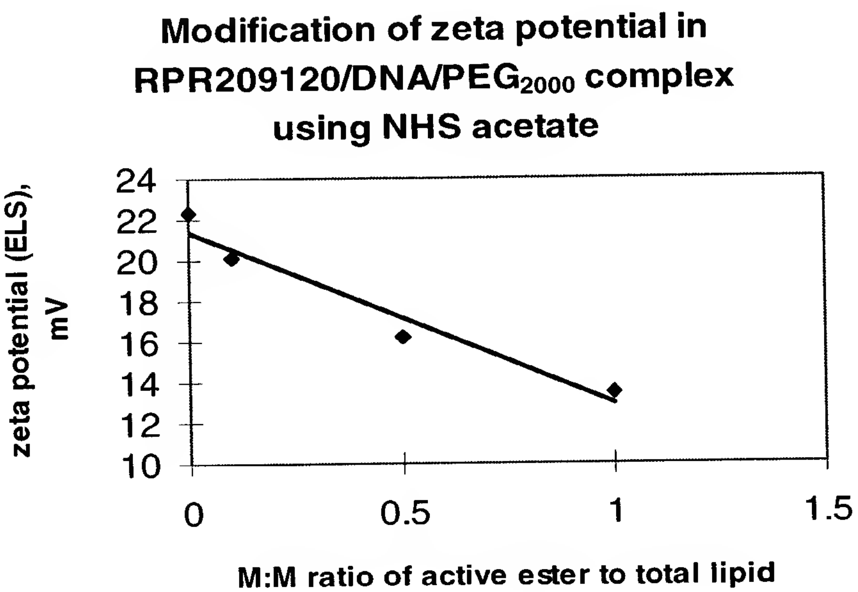

Figure 5. The NHS active ester, NHS-acetate, will react with RPR209120 to acetylate primary amines and reduce the surface charge, shown here by a reduction of the zeta potential. Zeta potential determined using a Coulter DELSA 440 electrophoretic light scattering (ELS) instrument.

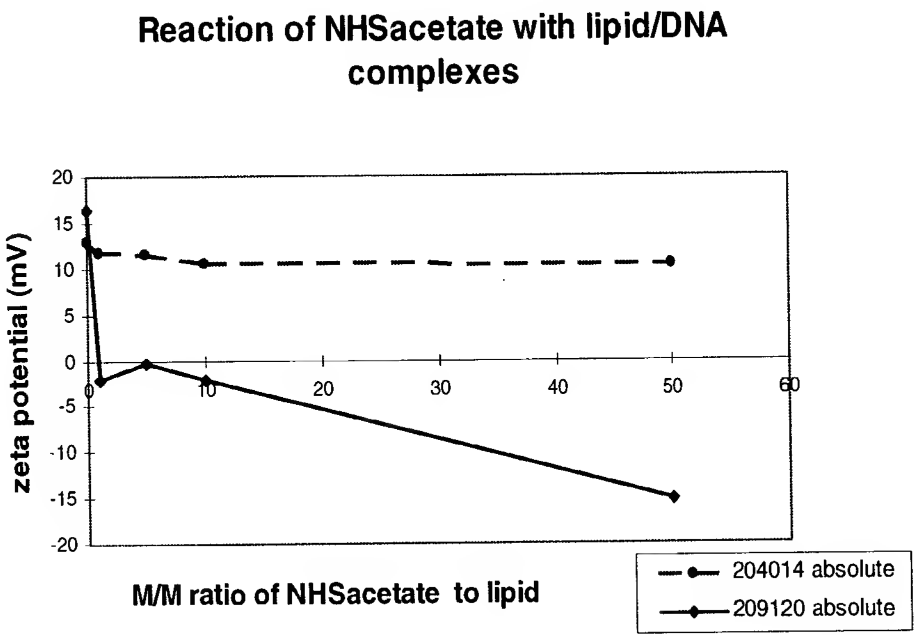

Figure 6. Complexes with RPR209120 (primary amine) react readily with NHS acetate, where RPR204014 (guanidinium and secondary amines) will not. Using high concentrations of the active ester, the zeta potential can be reduced to zero and even reversed.

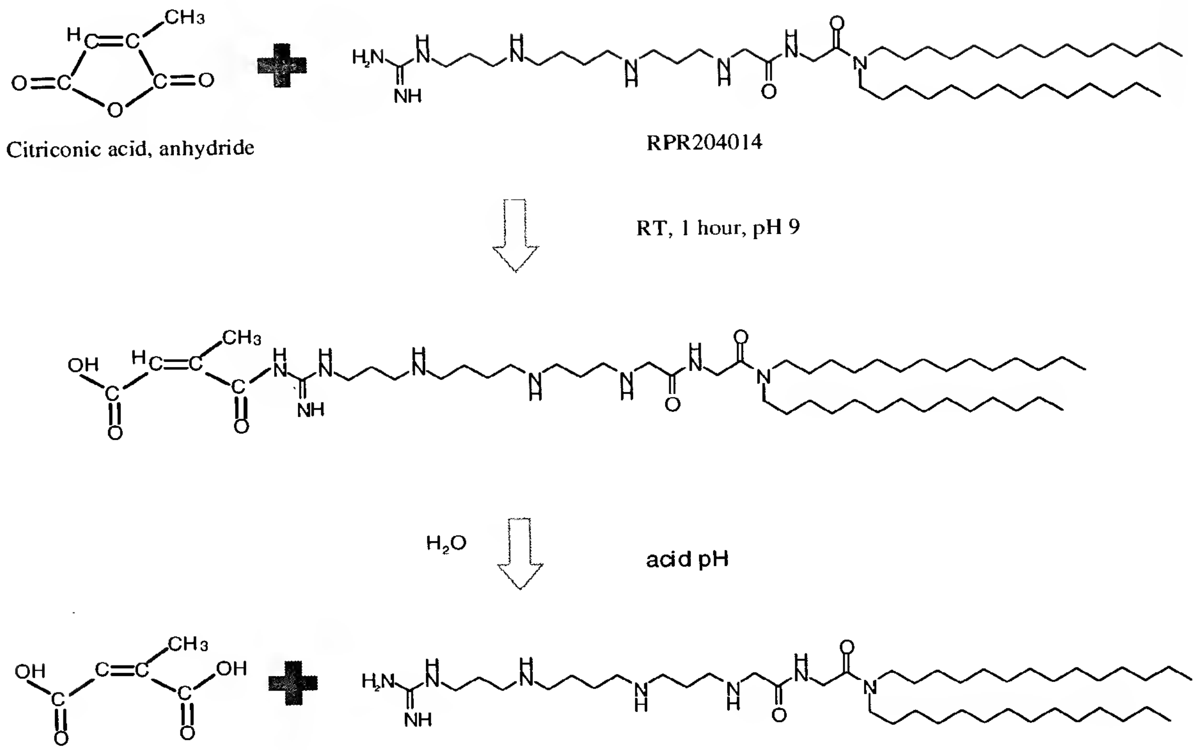

Figure 7. Chemistry of citraconic acid anhydride showing reversibility at low pH.

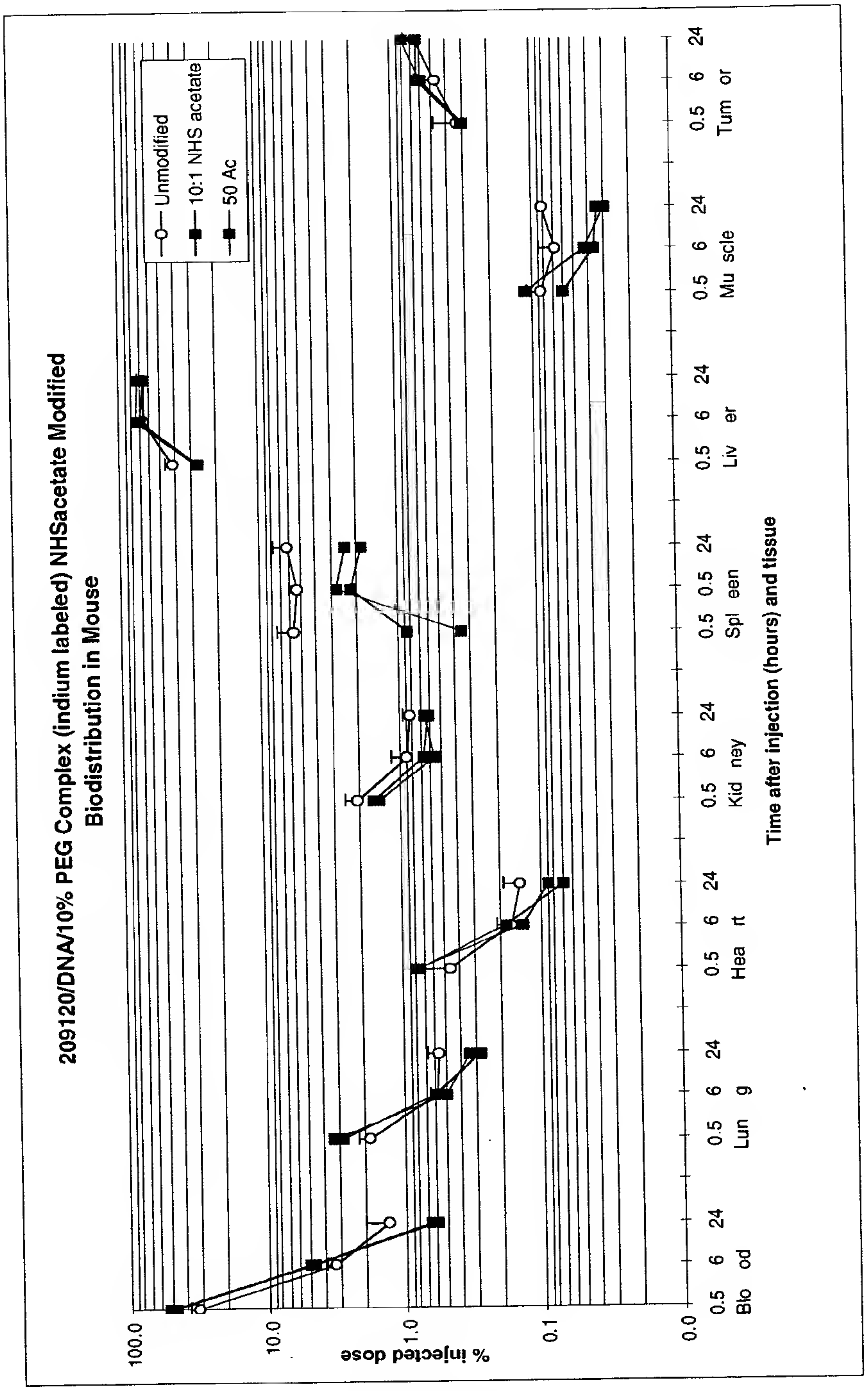

Figure 8. Composite graph showing biodistribution of NHS modified complex to various tissues.

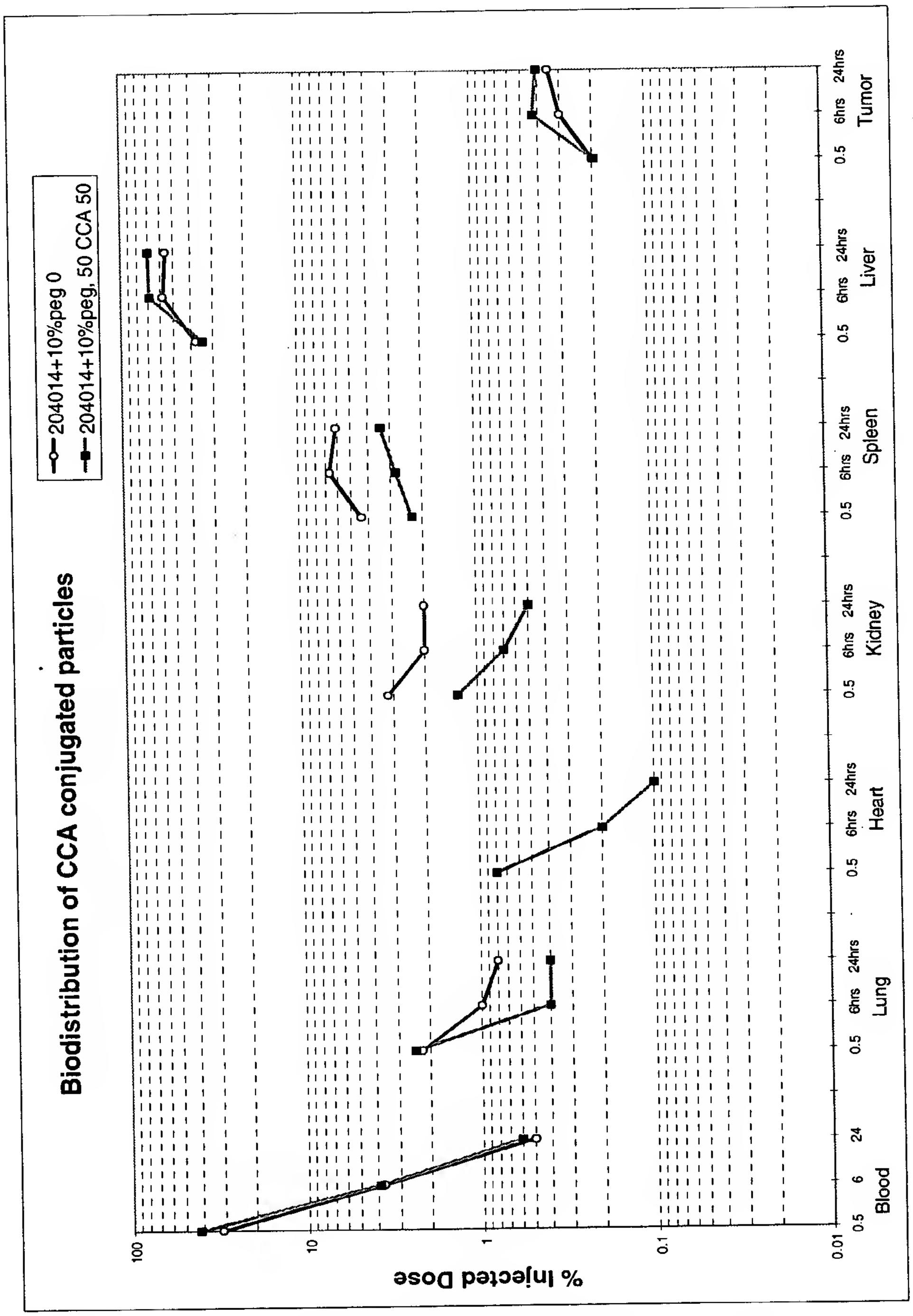

Figure 9. Biodistribution study using CCA modified complex.

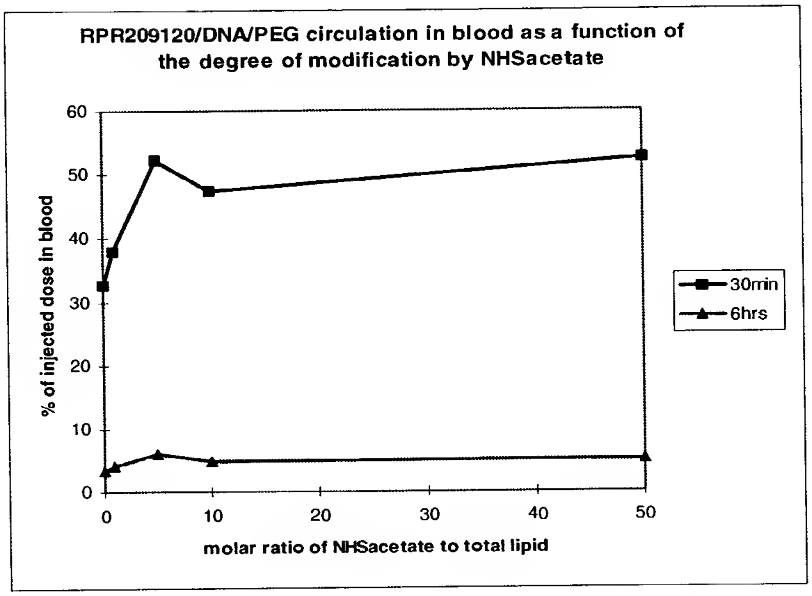

Figure 10. Circulation in blood as a function of time following IV injection and the amount of active ester used to react the particles. (Biodistribution in the mouse is tracked using the gamma emitter, 111Indium attached to the complex using a metal chelator-lipid conjugate. Complex is administered by tail vein injection. Mice are pre-injected subcutaneously with cultured tumor cells, 4T1, 10 to 14 days prior to testing.)

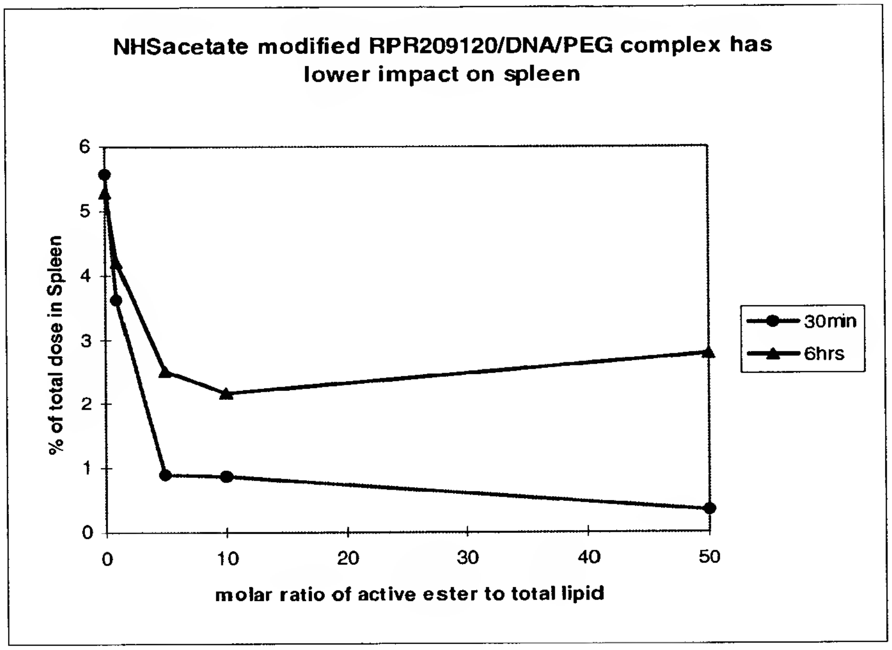

Figure 11. Reduced accumulation in spleen of modified complexes.

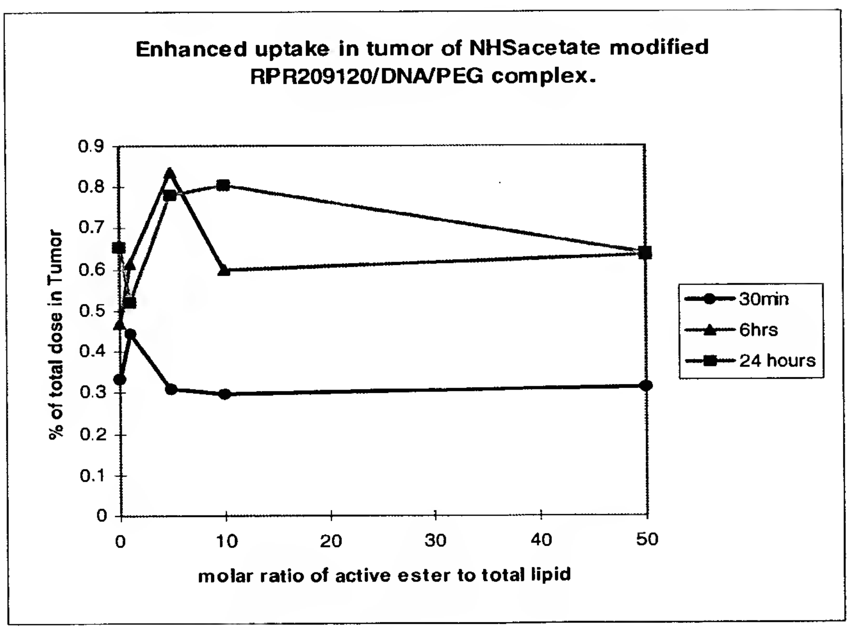

Figure 12. Improved uptake in tumor with charge modified complexes.

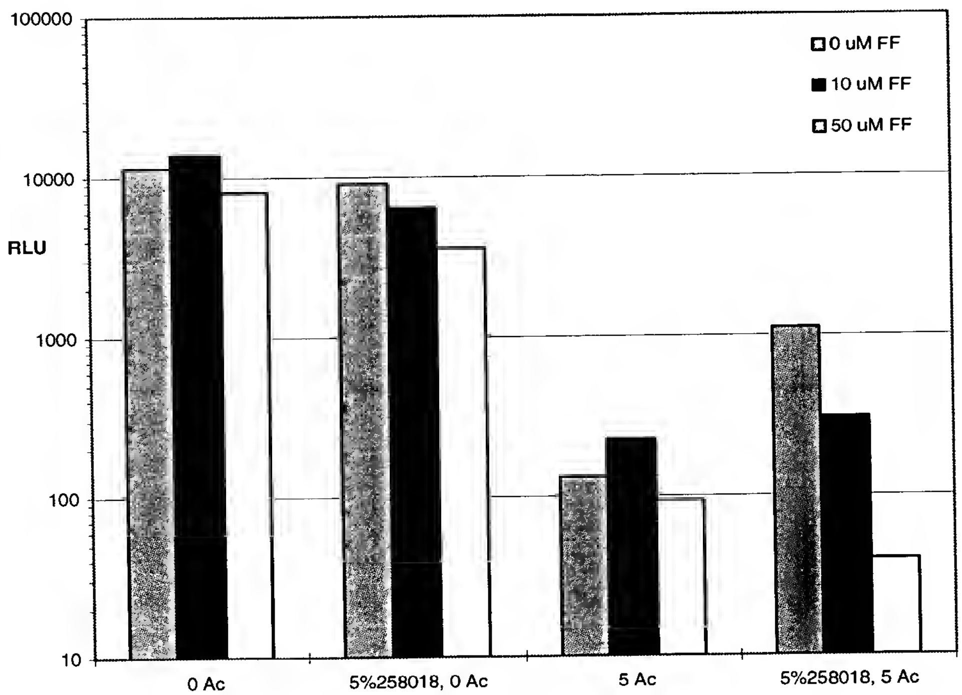

Figure 13. Folate mediated gene transfer in vitro of NHS-Acetate modified lipid DNA complex: 1 μg DNA + 5nmoles RPR209120 + 1nmole RPR204014 + 0.3nmole RPR 204293 w/wo 0.3nmole distearyl-PEG400-Folate (RPR258018), which was inserted after the complex was formed.

Figure 14. Expression of CAT transgene in different organs after IV injection of 100, 200, 400 or 800 μg DNA in chemically modified particles. Data are mean and individual values of four animals.